Research Highlights:

"Tit for Tat": FBXW7α targets C/EBPδ protein for degradation and inhibits pro-inflammatory signaling

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Tissue homeostasis depends on the ability of the organism to mount an inflammatory response to insult, injury or infection. Inflammation involves an assemblage of cooperating cells and signals that lays the groundwork for the repair of damage to the tissue. Normally once the healing process begins, the inflammatory process shuts off. In tumor progression however, chronic or acute exposure to noxious substances, infection, disease, anti-cancer therapy and the tumor itself can constitutively or inappropriately maintain the inflammatory process in the on state. Within tissues, chronic inflammation can combine with the evolutionary forces of mutation and natural selection to create the necessary conditions for the emergence of increasingly proliferative and invasive, metastatic cancer cells. A tumor in short, becomes "a wound that never heals," and the inflammation in the tumor and in its surroundings elicits an ever escalating cascade of messages sent at the wrong place and time to promote a permanent loss of tissue homeostasis and architecture (Dvorak, 1986 and Grivennikov et al, 2010).

In the last decade, the mechanisms by which inflammation contributes to carcinogenesis have become increasingly clear. Balamurugan *et al*, in a recent paper in Nature Communications show that there is a balance between two key molecules in macrophages (Balamurugan et al, 2013). The transcription factor CCAAT/enhancer binding protein delta (C/EBPδ) mediates and enhances inflammatory signaling in part by inhibiting the expression of the tumor suppressor FBXW7α (F-box and WD repeat domain containing protein 7 alpha). FBXW7α returns the "favor" by targeting C/EBPδ for degradation through the proteasome thereby shutting down the inflammatory response. FBXW7α has been shown previously to target several oncoproteins for degradation, but its role in inflammatory signaling is being worked out by Balamurugan and colleagues in the lab of Esta Sterneck at the NCI. In this study, Balamurugan *et al* demonstrate that C/EPBδ and FBXW7α regulate each other in an auto-feedback loop, which under normal conditions contributes to the stability of the system in response to inflammatory stimuli. In a tissue where cancer is arising, the loss of FBXW7α could contribute to constitutive activation of the inflammatory signaling, further promoting tumor evolution. Previously, C/EPBδ was shown to be downstream of Toll-like receptor-4 (TLR4) and functions as an amplifier of lipopolysaccharide (LPS) signaling (Litvak et al, 2009). However, this study demonstrates that C/EPBδ could also act as an upstream regulator of TLR4 expression. These findings suggest that C/EPBδ is not only an amplifier, but also supports the initiation of LPS signaling.

Using primary peritoneal mouse macrophages, primary human monocytes and monocyte/macrophage cell lines, they show that these cells selectively express the FBXW7α isoform, and that its expression in macrophages is downregulated by the inflammatory signaling cascade elicited by LPS and mediated by C/EBPδ. This shows that in addition to its tumor suppressor function that FBXW7 functions as a suppressor of inflammatory signaling. When FBXW7α expression is inhibited by C/EBPδ, it

promotes HIF-1α expression and HIF-1α mediated inflammatory responses. Furthermore, temporarily reducing the levels of FBXW7α in mice is sufficient to increase the serum levels of IL-6 in the mouse, a major driver of chronic inflammation and cancer risk, indicating that FBXW7 depletion is sufficient to elicit pro-inflammatory responses.

Completing the auto feedback loop, FBXW7α itself regulates C/EBPδ. FBXW7α physically interacts with C/EBPδ and is required for ubiquitination and degradation of C/EBPδ by the proteasome in macrophages. Less C/EBPδ in human and mouse cells results in attenuated inflammatory signaling confirming that C/EBPδ is a key regulator of pro-inflammatory signaling. In addition, there is cooperation between FBXW7α and GSK-3β to negatively regulate inflammation. GSK-3β phosphorylates C/EBPδ leading to its interaction with FBXW7α and subsequent ubiquitin-dependent degradation. Therefore, inhibition of GSK-3β activity through LPS signaling leads to stabilization of C/EBPδ. This suggests that GSK-3β and FBXW7 control the magnitude and duration of inflammatory signaling, and that loss of their function in a tissue will contribute to chronic inflammation and tumor promotion. However, previous studies have shown that GSK-3β activity contributes to chronic inflammation (Hofmann et al, 2010). Presumably, FBXW7α expression is inhibited under these conditions, suggesting that the role of GSK-3β in inflammatory signaling depends on the presence or absence of FBXW7α. Therefore, further mechanistic insights into the negative feedback loop between FBXW7α and C/EBPδ may identify molecules that can serve as targets for small molecule anti-inflammatory drugs and possibly even cancer therapeutics.

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